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Phytochemical and pharmacological studies on *Scorzonera alexandrina* Boiss

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Abstract The present study was designed to investigate secondary metabolite contents of *Scorzonera alexandrina* growing in the North–Western coast of Egypt. In addition hepatoprotective activity, reduction in blood sugar and ulcerative colitis were also evaluated. Chromatographic methods and spectroscopic analysis were used for isolation and identification of the compounds. In the hepatoprotective activity; twenty-four adult male Wistar albino rats were divided into four groups of six animals each. Rats of the 1st (normal control) and 2nd (CCl₄-intoxicated control) groups received the vehicle in a dose of 5 mL/kg. The 3rd and 4th groups were treated with the ethanol extract of *S. alexandrina* in doses of 200 and 400 mg/kg, respectively. In the ulcerative colitis study, the same extract was administered orally (200 and 400 mg/kg) to rats once a day for 5 consecutive days and the last dose was administered 2 h before induction of colitis by intra-rectal infusion of acetic acid. The obtained results revealed that *S. alexandrina* contains scopoletin, xanthotoxin, apigenin, luteolin, quercetin-7-*O*-rhamnoside and luteolin-7-*O*-glucoside, also it showed a significant decrease in blood glucose level, liver functions (ALT, AST, TP), also a significant anti-ulcer effect.

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1. Introduction

The genus *Scorzonera* encompasses about 160 species and being Ancient Mediterranean in origin, it is widely spread in arid regions of Eurasia and Africa, in Egypt, the genus *Scorzonera* is represented by five species, namely; *Scorzonera pseudo-*

lanata Grossheim, *Scorzonera mollis* Bieb, *Scorzonera schweinfurthii* Boiss, *Scorzonera drarii* Tackh and *Scorzonera alexandrina* Boiss. The first four species are very rare in the Egyptian deserts while the last species is very common and endemic to the North–Western Coastal strip of Egypt. *S. alexandrina* is a small perennial herbaceous plant with tuberous edible root. The flowers are pale mauve, deep purple at the center emitting a nice scent of vanilla. It appears only on rainy seasons (Täckholm, 1974).

Previous chemical investigations of this genus yielded dihydro-isocoumarins, flavonoids, lignans, phenolic acids, sesquiterpene, sesquiterpene lactone, triterpenes and a new class of bibenzyl derivatives (Zidorn et al., 2003; San et al., 2007). In addition to utilizing the fresh leaves as an ingredient in green salad, its sub aerial parts are considered to be a potent tonic

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and continue to be used in traditional medicine as analgesic, antirheumatic, anthelmintic, curing fever, carbuncle, mastitis and treatment of fertility, also it is used as a diabetic diet for containing inulin and laevulin (Baytop, 1984).

A new friedoolean-type triterpene, 3 β -acetoxyglutin-5(10)-en-6-oxo, together with seventeen known compounds were isolated from the roots of *Scorzonera austriaca* (Wu et al., 2011). Extracts prepared from the aerial parts of *Scorzonera cana* var. *jacquiniana*, *Scorzonera cana cinerea*, *Scorzonera cana eriophora*, *Scorzonera cana incisa* and *Scorzonera cana parviflora* showed a significant inhibitory effect on carrageenan and PGE₂-induced hind paw edema model as well as on p-benzoquinone-induced abdominal constriction test (Akkol et al., 2012).

No phytochemical and biological studies have been reported for *S. alexandrina*. Therefore, the present study was designed to investigate the phytochemical and biological activities of this plant.

2. Material and methods

2.1. Plant material

S. alexandrina was collected in the wild form from the North–Western coast of Egypt during spring (2010) and a voucher specimen has been deposited in the herbarium of the Desert Research Center, Cairo-Egypt. The collected plant was dried under shade and then grinded to fine powder.

2.2. Extraction and isolation

About 500 g of the air dried powder of *S. alexandrina* (aerial and sub aerial parts) were extracted by aqueous ethanol. The combined ethanolic extracts were concentrated under reduced pressure at a temperature not exceeding 35 °C. The ethanolic extract of *S. alexandrina* (25 g) was separated into fractions on a silica gel (500 g) column and eluted with Chloroform (fractions 1–12), after that Chloroform–methanol (fractions 13–45). Fractions 1–12 (1.8 g) were reappplied to a silica gel column eluted with chloroform, from which compounds 1 and 2 were isolated. Fractions 13–45 (6.5 g) were reappplied to a second silica gel column and eluted with chloroform:methanol from this column, compounds 3, 4, 5 and 6 were isolated.

2.3. Pharmacological studies

2.3.1. Experimental animals

Male Wistar albino rats (220–240 g) and albino mice of both sexes (25–28 g) were maintained in the Laboratory Animal Unit of the College of Pharmacy, Salman Bin Abdulaziz University. They were housed in polypropylene cages and fed with standard chow diet and water *ad libitum*. Animals were allowed to adapt to the laboratory environment for one week before experimentation. The care and handling of the animals were in accordance with the internationally accepted standard guidelines. All animal procedures were approved by an institutional review board of Pharmacy College, Salman Bin Abdulaziz University, KSA.

2.3.2. Acute toxicity experiment

Albino mice were divided into control and test groups (6 animals each). Control group received the vehicle (3% Tween 80)

while the test groups got graded doses (1000–4000 mg/kg) of *S. alexandrina* ethanol extract orally and were observed for mortality till 48 h and the LD₅₀ was calculated (Ghosh, 1994).

2.3.3. Effect of prolonged administration

Eighteen male Wistar albino rats were randomly divided into 3 groups of 6 animals. The 1st group was kept as control (5 mL/kg of 3% Tween 80), while 2nd and 3rd groups were administered the ethanol extract of *S. alexandrina* in doses of 200 and 400 mg/kg, respectively. All medications were administered orally with the aid of an orogastric cannula for 35 consecutive days. Rats were maintained under identical conditions with food and water *ad libitum* for the entire period with close observation. At the end of the experimental period, blood samples (2 mL) were drawn by puncturing retro-orbital venous sinus of each rat (under ether anesthesia) and centrifuged at 10,000 rpm for 5 min. Sera were separated to be used for the biochemical estimations.

2.3.4. Experimental induction of hepatic damage

CCl₄ was dissolved in corn oil in the ratio 1:1 v/v. Liver damage was induced in rats following subcutaneous (SC) injection of CCl₄ in the lower abdomen at a dose of 3 mL/kg (Theophile et al., 2006).

2.3.5. Hepatoprotective activity

Twenty-four adult male Wistar albino rats were randomly divided into four groups of six animals each. Rats of the 1st (normal control) and 2nd (CCl₄-intoxicated control) groups received the vehicle in a dose of 5 mL/kg. The 3rd and 4th groups were treated with the ethanol extract of *S. alexandrina* in doses of 200 and 400 mg/kg, respectively. All medications were administered orally by gastric intubation for 7 consecutive days. Two h after the last dose, normal control rats were given a single dose of corn oil (3 mL/kg, SC), while animals of the 2nd–4th groups received a single dose of CCl₄ (3 mL/kg, SC).

2.3.6. Measurement of liver and kidney function markers

Liver functions were evaluated by measuring the serum activity of alanine transaminase (ALT) and aspartate transaminase (AST), following the method of Reitman and Frankel (1957). The serum concentrations of total bilirubin (TB) (Walter and Gerarde, 1970), total protein (TP) (Henary et al., 1974) and albumin (Alb) (Doumas et al., 1971) were estimated. Serum levels of urea (Wills and Savory, 1981) and creatinine (Kroll et al., 1987) were determined colorimetrically as measures of kidney functions.

2.3.7. Measurement of blood glucose level

Determination of the blood glucose level was done by the glucose-oxidase principle (Rheney and Kirk, 2000), results were reported as mg/dL.

2.3.8. Effect on ulcerative colitis

The total ethanolic extract of *S. alexandrina* and the standard; dexamethasone (DEX) was suspended separately in 3% v/v Tween 80 (vehicle).

Thirty male Wistar albino rats were divided into 5 equal groups. Groups 1 and 2 (normal and colitis control groups,

respectively) were given the vehicle in a dose of 5 mL/kg. Group 3 (reference group) was given DEX in a dose of 0.2 mg/kg. Groups 4 and 5 were administered the ethanolic extract of *S. alexandrina* in doses of 200 and 400 mg/kg, respectively. All medications were administered orally via the aid of an orogastric cannula, once a day for 5 consecutive days and the last dose was administered 2 h before colitis induction.

2.3.9. Induction of ulcerative colitis

Rats were fasted for 24 h with access to water *ad libitum* after which they were lightly anesthetized with ether. A polyethylene catheter with 2 mm diameter was inserted through the rectum into the colon to a distance of 8 cm (Ghaneya and Soliman, 2010). For Ulcerative colitis induction, 2 mL of 4% (v/v) acetic acid was infused into the colon of all rats (except the normal control group) through the catheter, held in place for 30 s, and then flushed with 5 mL of phosphate buffer solution; pH = 7. The catheter was left in place for few seconds then gently removed.

2.3.10. Assessment of colonic lesions

Two days after the induction of colitis, each rat was inspected and diarrhea was recorded. Rats were sacrificed using ether anesthesia and colonic segments (8 cm in length and 3 cm proximal to the anus) were excised, opened along its mesenteric border, and rinsed thoroughly in ice-cold normal saline. The colon specimen of each rat was weighed and the wet weight/length ratio was calculated as ratio of the colon specimen weight vs. its length (mg/cm). It was used as a parameter to assess the degree of colon edema, which reflected the severity of colitis. The specimens were examined under a dissecting microscope and all visible damages were evaluated using the scoring system reported by Morris et al. (1989) with some modifications. The lesion scores were: 0 = no damage, 1 = local edema and inflammation without ulcers; 2 = one ulcer without inflammation; 3 = one to two ulcers with inflammation and lesion diameter < 1 cm; 4 = More than two ulcers with lesion diameter 1–2 cm; 5 = Severe ulceration with lesion diameter > 2 cm. **Ulcer area** was measured for each specimen using a 1 mm² grid. **Ulcer index** was measured by summing the lesion score and the ulcer area for each colon specimen (Minaiyan et al., 2006).

3. Statistical analysis

The values are expressed as mean \pm standard error of six observations in each group. All groups were subjected to one-way analysis of variance (ANOVA), which was followed by Bonferoni's test to determine the intergroup variability by using SPSS ver. 14.

4. Results

4.1. Toxicological Studies

S. alexandrina in oral doses up to 4000 mg/kg b. wt. did not produce any demonstrable acute toxic effects or deaths in all groups of animals.

4.2. Effect of prolonged administration

From the data presented in Table 1 it can be concluded that *S. alexandrina* showed a reduction in blood glucose after prolonged administration in both doses 200 and 400 mg/kg, also the same trend at 400 mg/kg was achieved in ALT (61.70 ± 3.82 U L⁻¹), T. Bilirubin (1.53 ± 0.11 mg d L⁻¹) and T. Protein (8.5 ± 0.38 mg d L⁻¹).

4.3. Hepatoprotective activity

S. alexandrina showed significant effects on liver function tests Table 2 which appeared as a reduction of all studied parameters at dose of 400 mg/kg, also a significant decrease in blood glucose (188.5 ± 7.11 mg d L⁻¹ vs. 221.7 ± 9.06 mg d L⁻¹ of CCl₄-intoxicated Control) was observed in CCl₄ induced-hepatotoxicity experiment.

4.4. Anti-ulcerogenic effect

Experimental colitis was accompanied by marked anorexia, prostration, hypomotility and piloerection after acetic acid challenge (data not shown). Diarrhea, as evidenced indirectly by perianal fur soiling, was prominent among colitic animals. The incidence of diarrhea, lesion score, ulcer area and ulcer index were used as the indicators for the effectiveness of the tested extracts against colitis induced by acetic acid in rats.

The intestinal damage induced by acetic acid was associated with a significant increase of wet weight/length ratio of the colon specimens as an indicator of inflammation. The wet weight/length ratio increased 3-fold in rats with acetic acid colitis compared to normal rats (0.97 ± 0.04 vs. 0.30 ± 0.02 g/cm). This ratio was greatly improved in rats that pre-medicated with 0.2 mg/kg of DEX (0.36 ± 0.02 g/cm) and 400 mg/kg of the ethanolic extracts of *S. alexandrina* (0.42 ± 0.01 g/cm). Morphologically, a thickened wall and brown to black lesions were observed in the injured colon specimens of control colitis rats, 2 days after rectal infusion of acetic acid. Under dissecting microscope, hyperemia, edema, erosion, and severe ulceration were also observed. Control

Table 1 Effect of prolonged oral administration of ethanolic extract of *S. alexandrina* for 35 days on the serum activity of ALT and AST and serum levels of total bilirubin, total protein, albumin and glucose in rats, (n = 6).

Groups	Doses (mg/kg)	ALT (U L ⁻¹)	AST (U L ⁻¹)	T. bilirubin (mg d L ⁻¹)	T. protein (g d L ⁻¹)	Albumin (g d L ⁻¹)	Glucose (mg d L ⁻¹)
Control	00	66.50 \pm 3.56	139.38 \pm 6.44	1.51 \pm 0.10	8.0 \pm 0.34	3.5 \pm 0.17	115.83 \pm 5.84
<i>S. alexandrina</i>	200	63.50 \pm 1.50	135.38 \pm 5.41	1.51 \pm 0.2	8.2 \pm 0.30	3.4 \pm 0.12	109.83 \pm 4.94*
	400	61.70 \pm 3.82*	134.27 \pm 6.80	1.53 \pm 0.11*	8.5 \pm 0.38*	3.4 \pm 0.12	88.63 \pm 4.75**

* Significance ($p < 0.05$).

** Significance ($p < 0.01$).

Table 2 Effect of the ethanolic extract of *S. alexandrina* on the serum activity of ALT and AST and serum levels of total bilirubin, total protein, albumin and glucose in rats with CCl₄ induced-hepatotoxicity.

Groups	ALT (U L ⁻¹)	AST (U L ⁻¹)	T. bilirubin (mg d l ⁻¹)	T. protein (g d l ⁻¹)	Albumin (g d l ⁻¹)	Glucose (mg d l ⁻¹)
Normal control	68.0 ± 2.11*	140.5 ± 4.18*	1.24 ± 0.06*	8.47 ± 0.39*	3.65 ± 0.18*	115.83 ± 5.84*
CCl ₄ -intoxicated control	372.5 ± 11.9	434.6 ± 13.4	3.3 ± 0.11	5.7 ± 0.19	2.1 ± 0.14	221.7 ± 9.06
<i>S. alexandrina</i> (200 mg/kg) + CCl ₄	360.7 ± 9.7	419.2 ± 11.6	3.4 ± 0.14	5.8 ± 0.17	2.16 ± 0.12	214.5 ± 8.20
<i>S. alexandrina</i> (400 mg/kg) + CCl ₄	311.7 ± 9.3*	377.2 ± 13.9**	3.8 ± 0.15*	6.1 ± 0.13*	2.6 ± 0.13*	188.5 ± 7.11*

* Significance compared to CCl₄ group ($p < 0.05$).** Significance ($p < 0.01$).**Table 3** Effects of oral administration of ethanolic extract of *S. alexandrina* on the macroscopic parameters of ulcerative colitis induced by acetic acid in rats ($n = 6$).

Groups	Doses (mg/kg)	Lesion score (0–5)	Ulcer area (cm ²)	Ulcer index	Wet W/L ratio (g/cm)
Sham (Normal control)	00	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.30 ± 0.02
Colitis control	00	3.9 ± 0.20	5.0 ± 0.23	8.9 ± 0.25	0.97 ± 0.04
DEX	0.2	2.1 ± 0.15***	2.2 ± 0.18***	4.3 ± 0.25***	0.36 ± 0.02***
<i>S. alexandrina</i>	200	3.7 ± 0.18	4.7 ± 0.24*	8.4 ± 0.33	0.68 ± 0.03*
	400	3.5 ± 0.18*	4.1 ± 0.21*	7.6 ± 0.31*	0.42 ± 0.01**

* Significance ($p < 0.05$).** Significance ($p < 0.01$).*** Significance ($p < 0.001$).

colitis rats showed lesion score, ulcer area and *ulcer index* values of 3.9 ± 0.20 , $5.0 \pm 0.23 \text{ cm}^2$ and 8.9 ± 0.25 , respectively (Table 3). These inflammatory indices were significantly improved by oral dosing of DEX and ethanolic extracts of *S. alexandrina* (200 and 400 mg/kg) for 5 days prior to ulcer induction.

4.5. Phytochemical Studies

4.5.1. Isolated compounds

Compound (1): White crystals, ¹H NMR (500 MHz, CDCl₃) δ 7.90 (1H, d, $J = 12.5$, H-4), δ 7.24 (1H, s, H-5), δ 6.72 (1H, s, H-8), δ 6.24 (1H, d, $J = 10.5$, H-3) and δ 3.77 (3H, s, OCH₃). ¹³C-NMR (125 MHz, CDCl₃): δ 160.78 (C-2), 152.0 (C-7), 149.22 (C-9), 145.41 (C-6), 144.1 (C-4), 111.7 (C-3), 110.3 (C-10), 109.7 (C-5), 102.28 (C-8), 56.18 (–OCH₃).

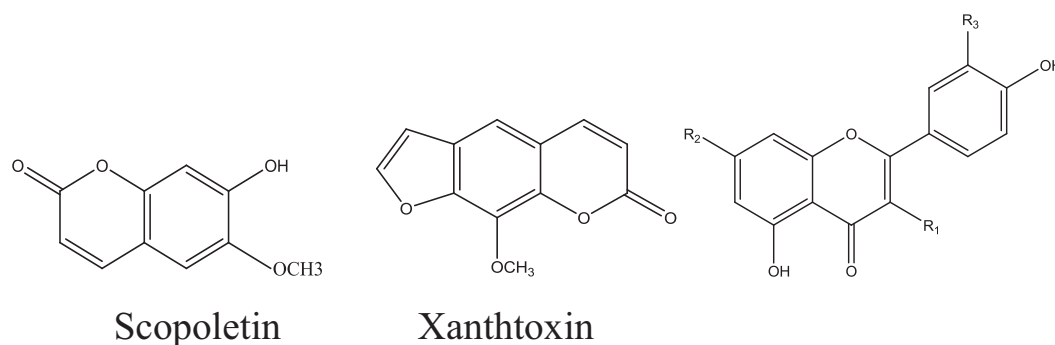
Compound (2): Obtained as needle crystals, ¹H-NMR (500 MHz, CDCl₃): δ 7.8 (1H, d, $J = 9$ Hz, H-4); 7.62 (1H, d, $J = 2.5$ Hz, H-5'). δ 7.35 (1H, s, H-5), δ 7.1 (1H, d, $J = 2.5$ Hz, H-4'); δ 6.38 (1H, d, $J = 9.5$ Hz, H-3). ¹³C-NMR (125 MHz, CDCl₃): 161.36 (C-2), 147.73 (C-7), 147.19 (C-2'), 145.32 (C-4'), 142.77 (C-4), 126.66 (C-6), 116.71 (C-5), 113.66 (C-3), 113.76 (C-5'), 106.62 (C-3'), 60.44 (C-8).

Compound (3): Yellow powder; ¹H-NMR (500 MHz, CD₃OD): δ 7.46 (1H, dd, $J = 8$, 2.3 Hz, H-6'), 7.42 (1H, d, $J = 2.4$ Hz, H-2'), 6.90 (1H, d, $J = 8.2$ Hz, H-5'), 6.67 (1H, s, H-3), 6.45 (1H, d, $J = 2.1$ Hz, H-6), 6.20 (1H, d, $J = 2.1$ Hz, H-8). ¹³C NMR (125 MHz, CD₃OD): δ 182.0 (C-4), 164.5 (C-5), 164.3 (C-2), 161.9 (C-7), 157.7 (C-9), 150.1 (C-4'), 146.1 (C-3'), 121.9 (C-1'), 119.3 (C-6'), 116.4 (C-5'), 113.8 (C-2'), 104.1 (C-10), 103.2 (C-3), 99.2 (C-6), 94.3 (C-8),

Compound (4): Yellow powder; (500 MHz, CD₃OD): δ 6.19 (1H, d, $J = 2.1$ Hz, H-8), 6.47 (1H, d, $J = 2.1$ Hz, H-6), 6.76 (1H, s, H-3), 6.92 (2H, d, $J = 9.7$ Hz, H-2' and H-6'), 7.92 (2H, d, $J = 9.7$ Hz, H-3' and H-5'). ¹³C NMR (125 MHz, CD₃OD): δ 182.1 (C-4), 164.5 (C-7), 164.1 (C-1'), 161.5 (C-4'), 161.8 (C-9), 157.7 (C-5), 128.8 (C-3 and C-5'), 121.6 (C-2), 116.3 (C-2' and C-6'), 104.1 (C-10), 103.2 (C-3), 99.2 (C-6) 94.3 (C-8).

Compound (5): yellow crystal, ¹H NMR (CD₃OD): δ 7.4 (1H d, $J = 2$ Hz H-2'), 7.3 (1H dd, $J = 8.5$ $J = 2.1$ Hz H-6'), 6.8 (1H d, $J = 8.5$ Hz H-5'), 6.4 (1H d, $J = 2.5$ Hz H-8), 6.2 (1H d, $J = 2.5$ Hz H-6), 5.2 (1H d, $J = 2.5$ Hz H-1 rhamnose), 1.2 (3H d, $J = 6$ Hz CH₃), sugar protons at δ 3.2–3.9. ¹³C NMR (CD₃OD) δ 178.9 (C-4), 163.20 (C-7), 159 (C-5), 155.20 (C-9), 149 (C-2), 146.70 (C-4'), 144.80 (C-3'), 134.80 (C-3), 121.30 (C-1'), 119.70 (C-6'), 115.60 (C-5'), 114.80 (C-2'), 103.20 (C-10), 100.70 (C-1" sugar), 99.80 (C-6), 92.50 (C-8), 75.20 (C-5"), 70.60 (C-4"), 70.30 (C-3"), 68.10 (C-2") and 17.60 (–CH₃).

Compound (6): Yellow powder; ¹H-NMR (500 MHz, DMSO-d₆): δ 7.43 (1H, s, H-2'), 7.41 (1H, d, $J = 8.0$ Hz, H-6'), 6.93 (1H, d, $J = 8.0$ Hz, H-5'), 6.83 (1H, d, $J = 1.8$ Hz, H-8), 6.64 (1H, s, H-3), 6.44 (1H, d, $J = 1.8$ Hz, H-6), 5.10 (1H, d, $J = 7.8$ Hz, H-1"), 3.85 (1H, dd, $J = 12.2$, 1.8 Hz, H-6"), 3.68–3.60 (1H, m, H-5"), 3.56 (1H, t, $J = 9.0$ Hz, H-3"), 3.49 (1H, t, $J = 9.0$ Hz, H-3"), 3.41 (1H, t, $J = 9.0$ Hz, H-4"). ¹³C-NMR (125 MHz, DMSO-d₆): δ 182.6 (C-4), 165.65 (C-7), 147.1 (C-30), 120.6 (C-10), 116.9 (C-5'), 113.5 (C-2'), 163.88 (C-2), 161.44 (C-5), 157.78 (C-9), 152.5 (C-4'), 120.2 (C-6'), 106.0 (C-10), 103.2 (C-3), 100.6 (C-1), 100.21 (C-6), 95.7 (C-8), 77.5 (C-3"), 76.66 (C-5"), 73.4 (C-2"), 70.2 (C-4"), 61.2 (C-6").



Compound	R1	R2	R3
Apegenin	H	OH	H
Luteolin	H	OH	OH
Luteolin-7-O-glucoside	H	O-glucoside	OH
Quercetin-7-O-rhamnoside	OH	O-rhamnoside	OH

Figure 1 Isolated compounds from *S. alexandrina*.

5. Discussion

5.1. Effect of prolonged administration

From the data presented in Table 1 it can be concluded that *S. alexandrina* showed reduction in blood glucose after prolonged administration in both doses 200 and 400 mg/kg, also the same trend at 400 mg/kg was achieved in ALT ($61.70 \pm 3.82 \text{ U L}^{-1}$) also T. Bilirubin ($1.53 \pm 0.11 \text{ mg d l}^{-1}$) and T. Protein ($8.5 \pm 0.38 \text{ g d l}^{-1}$). These are in the same trend with the previous review (Baytop, 1984), and no significant effects were observed in urea and creatinine levels. Urea and creatinine are the most sensitive biochemical markers employed in the diagnosis of renal damage. In kidney damage, there will be retention of urea and creatinine in the blood (Nwanjo et al., 2005), therefore a marked increase in serum urea and creatinine is indications of functional damage to the kidney (Panda, 1999). By these indicators, ethanol extract of *S. alexandrina* is therefore, not nephrotoxic in rats.

5.2. Hepatoprotective activity

Liver marker enzymes are localized in the cytosol of hepatic cells and thus are extruded into the serum when cells are damaged or necrotic. In this study, rats intoxicated with CCl_4 developed significant hepatic damage as manifested by a significant increase in the serum activities of ALT and AST that are indicators of hepatocyte damage and loss of functional integrity. Pretreatment of rats with the ethanol extract of *S. alexandrina* in dose of 400 mg/kg effectively protected rats against CCl_4 -induced hepatic damage, resulting in reduction in serum activities of liver marker enzymes when compared to the intoxicated control rats. Decrease in the level of these enzymes with *S. alexandrina* is an indication of the stabilization of plasma membrane as well as the repair of liver damage caused by CCl_4 .

Further, the rise in the level of TB in serum following CCl_4 intoxication is also a measure of hepatotoxicity and could be

attributed to impaired hepatic clearance due to hepatic parenchymal damage and biliary obstruction (Blanckaert and Schmid, 1982). The ability of the ethanol extract of *S. alexandrina* (400 mg/kg) to reduce the level of TB in the serum of intoxicated rats suggests its potential hepatoprotective effect. The lowered serum levels of TP and Alb due to CCl_4 -intoxication is attributed to the initial damage of the endoplasmic reticulum which results in the loss of P-450 leading to fatty liver (Recknagel, 1967). Administration of the ethanol extract of *S. alexandrina* in dose of 400 mg/kg remarkably prevented CCl_4 -induced reduction of TP and Alb in serum. This assures the hepatoprotective activity of this extract against damage by CCl_4 . This activity may be attributed to flavonoid contents of this plant. In this trend Jin et al. (2011) reported the flavonoid rich extract of *Salvia plebeian* and its striking beneficial effects in preventing carbon tetrachloride induced cute hepatic injury in mice. The contents of *Salvia plebeian* were mainly flavonoids which have been identified as hispidulin, homoplantagin, nepetin, luteolin luteolin-7-glucoside and nepetin-7-glucoside.

5.3. Anti-ulcerogenic effect

The present study demonstrated that treatment of rats with the ethanolic extract of *S. alexandrina* (200 and 400 mg/kg) reduced the inflammation and the acute colonic damage induced by acetic acid. The protective effect against acetic acid induced ulcers could be attributed to the phenolic and/or flavonoid content of the tested plant and their reactive oxygen species scavenging property. These results are in agreement with that of Akkol et al. (2011) who revealed that, methanol-water extracts from aerial parts of *Scorzonera latifolia* and *S. mollis* were found to have wound healing and anti-inflammatory activities compared to control groups. This might be due to the combined effect of the constituents present in the studied plant; the highest anti-inflammatory activity was seen on the extract of *S. latifolia*, which has the highest amount of hyperoside. Luteolin-7-O-glucoside was found to possess anti-inflam-

matory activity (Akdemir et al., 2011). Therefore, anti-ulcerogenic effect (anti-inflammatory) potential of *S. alexandrina* may be attributed to the phytoconstituents (Luteolin and luteolin-7-O-glucoside) present in this plant, which may be either due to their individual or additive effect that speeds up the process.

5.4. Phytochemical studies

5.4.1. Isolated compounds

Compounds **1** and **2** (Fig. 1) were isolated from chloroform fractions. They were identified as scopoletin and xanthotoxin by comparing their TLC chromatograms, ¹H NMR and ¹³C NMR spectra with reference samples and published data (Khamis et al., 1999; Murray et al., 1982).

From the present and published data (Lin and Harnly, 2010; Lee et al., 2013) compound **3** was identified as Luteolin.

Compound **4** was identified as apigenin (Lin and Harnly, 2010; Lee et al., 2013)

Acid hydrolysis (Harborne et al., 1975) of compound **5** revealed the sugar rhamnose, which was identified by TLC (ethyl acetate-methanol-acetic acid-water (65:15:10:10). The aglycone was identified based on comparison by TLC and UV shift reagents. Compound **5** was substituted at position 7 as indicated by their UV spectra. From these data and published data (Mabry et al., 1970) identified these compounds as quercetin 7-O-rhamnoside.

Acid hydrolysis of compound **6** revealed that the glycone moiety was glucose, which was identified by TLC (ethyl acetate-methanol-acetic acid-water (65:15:10:10). The aglycone was identified based on comparison by TLC and UV shift reagents and it was identical with compound **3**.

From given and published data (Lee et al., 2013) compound **6** was identified as Luteolin-7-O-glucoside.

6. Conclusion

In the present study, it has been observed that *S. alexandrina* offered six compounds namely, Scopoletin, xanthotoxin, apigenin, luteolin, luteolin-7-O-glucoside, quercetin-7-O-glucoside also this plant showed hepatoprotective and anti-ulcerogenic activities, which were mainly due to the flavonoid contents of this plant.

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